CHLOROFORM TODAY:

The Modern Afterlife of an Early General Anesthetic

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INTRODUCTION

Although chloroform ($CHCl_3$) disappeared as a human anesthetic after World War II, it is still very much with us today. Chloroform is widely used in the chemical and pharmacological industries, in veterinary medicine for anesthesia and euthanasia and in dentistry for endodontic prosthetics. Chloroform continues to be used in suicides, attempted thefts, homicides and abductions, and as a pleasure inducer and erotic enhancer (occasionally with fatal sequels). Novels, films and the Internet often describe its administration for criminal or sexual purposes. Recently $CHCl_3$ has also raised much interest as a potential pollutant of our environment: over 500 publications have addressed that topic. Using the reviews cited, this paper offers an overview of many of these continued uses and concerns.

CHCL3 FOR INDUSTRY

Developed countries manufacture large amounts of CHCl3. The five main US producers make 800 million pounds of CHCl3 a year, about one third of the world's output. The US now exports 240 pounds annually. The American CHCl3 is made by hydrochlorination of methanol; overseas it is generally produced by oxychlorination of methane. This manufacture takes place in a closed system and very little CHCl3 is thus released into the atmosphere.

In the U.S. 90 to 98% of the CHCl3 is used to manufacture the fluorocarbon HCFC 22, a refrigerant for air conditioners and freezers and a propellant for aerosols and fire extinguishers. Although very little HCCF 22 reaches the stratosphere or depletes the ozone, it will be phased out in 2020.

The remaining 2 to 8% CHCl3 (10 million tons a year) is employed in the U.S. as a solvent and extractor of fats, resins, waxes and lacquers and as a component of dyes, polishes, photographic products and plastics and as pesticide and fumigant for stored grain. All those processes occur in closed systems and are supervised by OSHA. Thus

little CHCl3 is released and endangers the workers in the U.S. Serious industrial accidents have occurred overseas, however.

In the U.S. 675 tons CHCl3 are used annually in manufacturing and processing pharmaceuticals, especially extracting and purifying antibiotics. Since July, 1976 the U.S. food and Drug Administration has banned CHCl3 as an additive to drugs, cosmetics and hygiene products but allows traces of CHCl3 resulting from drug processing.

In 1991 the U.S. National Institute of Occupational Safety and Health estimated that 96,000 American workers were exposed to industrial CHCl3 each year but found few, mostly benign, symptoms such as dizziness, lassitude, irritation, headaches, nausea and mouth dryness. Cases of severe hepatitis have been reported in foreign, poorly regulated plants. Female technicians working with solvents containing CHCl3 (but also other compounds) in a Swedish medical laboratory showed a slight increased rate of spontaneous abortions.

In 1978 OSHA proposed a CHCl3 limit of 50 ppm/ 8 hour exposure for American plants with a gradual decrease to an ideal 2 ppm in the future. U.S. plants have an average concentration of 6 ppm. OSHA has

published strict regulations to prevent accidental inhalation or skin burns and guidelines for their treatment.

ACUTE CHCL3 TOXICITY

The literature continues to report severe, often fatal, cases of acute poisoning CHCl3 ingested for suicide or homicide or inhaled for recreation. The survivors, especially after ingestion, generally develop liver fatty infiltration or centro-lobular necrosis and damage to the renal proximal tubules. Similar hepato-renal lesions were described in the 1970-1980 studies in which the animals were gavaged with large doses of CHCl3 diluted in oil. Animals inhaling CHCl3 also developed necroses of the nasal mucosa. Liver and renal function tests showed marked impairment. These studies revealed marked variations in the degree of damage according to species, strain, sex and mode of administration. Those large variations and the high doses and prolonged periods of CHCl3 administration militate against extrapolating these results to man.

CHCL3 KINETICS AND METABOLISM

Whole body autoradiography, developed in the 1960s and 1970s, has contributed to our knowledge of the distribution of CHCl3 in animals. There exist very few human kinetic studies and the large variations found in animals according to species, sex and metabolism suggest caution in extrapolating these results to man.

Substantial amounts of CHCl3 are quickly absorbed through the lungs, the gastro-intestinal tract and the skin. The absorption of ingested CHCl3 is potentiated by its oil solvent, with marked first phase effect and accumulation in the liver. A similar effect occurs in the lung after inhalation. Studies with radio-labeled CHCl3 have confirmed the early 1900s findings that CHCl3 is rapidly and significantly taken up by the fat-rich viscera (especially brain, liver and kidney), the fat and the muscle mass and that it easily crosses the placenta.

A Swedish worker in 1983 found that CHCl3 is selectively taken up by specific receptors in the cerebellar cortex. If confirmed, these findings may explain some cerebellar symptoms seen in CHCl3 addicts(ataxia, dysarthria, tremors) Whatever its mode of administration, CHCl3 is

largely exhaled, mainly unchanged although a small amount is metabolized to C02 which is exhaled and Cl- ions which are excreted through the kidneys.

The extensive works of LR Pohl et al in the U.S. and H. Uehleke in Germany have shown that small quantities of the absorbed CHCl3 are metabolized into phosgene (C0Cl2) in the liver and, most probably, also in the kidney. Inhaled CHCl3 may also undergo that transformation in the nasal mucosa.

With a few exceptions, the C0Cl2 hypothesis has been accepted although it is likely that other, less important, pathways also exist. In the liver lobule and, most probably, in the renal cortex, CHCl3 undergoes oxidative cleavage of the C-H bond, a reaction catalyzed by the cytochrome P 450 and NADPH oxidoreductases. This produces the unstable trichloromethanol which is immediately chlorinated into C0Cl2. This C0Cl2 is quickly hydrolyzed into C02 and HCl. Most C02 is exhaled but a small part is converted into NaHC03 & excreted through the kidney. The HCl is neutralized in the plasma although some residual may dam age the cell. C0Cl2 is highly neutrophilic and irreversibly binds covalently with the nucleophilic SH, OH and NH radicals of the

microsomal and, to a lesser degree, mitochondrial macromolecules of the proteins and lipids and thus damages or destroys the cell. C0Cl2 is thus the main hepato-renal toxin. C0Cl2 also binds with the SH radicals of gluthatione and cystein. These protoplasmic components thus protect the cell unless overcome by an excess of C0Cl2. Several recent studies found C0Cl2 binding to the nuclear DNA and RNA. If confirmed, these findings would suggest that CHCl3 may be a mutagen.

The production of C0Cl2 is an aerobic process occurring in well oxygenated tissues. However, in animals whose microsomal hepatic enzymes have been stimulated by pre-treatment with phenobarbital or ethanol and which receive large doses of CHCl3, some of that CHCl3 undergoes anaerobic reduction to produce dichloro-methyl radicals CHCl2 and cytotoxic HCl and C0. CHCl2 may induce lipid peroxidation and thus damage the cell membrane and protoplasm. This minor pathway, occurring in poorly oxygenated areas such as the center of the hepatic lobule, may explain the centro-lobular necroses described after CHCl3 anesthesia in the early 20th century.

C0Cl2 production is potentiated by pre-treatment with phenobarbital, ethanol or ketones with increased hepato-renal

damage. Newborn animals with low microsomal cytochrome oxidase content are resistant to ChCl3.

The above findings confirm the early 1900s claims of Whipple and the John Hopkins University researchers that cystein feeding and young age protected their dogs from CHCl3 damage whereas fasting and alcohol increased the latter. They also support Lucas' hypothesis (1928) that the toxicity of CHCl3 is due to its metabolites.

The production of C0Cl2 in the kidney is less evident than in the liver; some researchers rule out the kidney as a source of C0Cl2 and suggest that the tubular cell damage is caused by C0Cl2 produced elsewhere, e.g. in the liver. The large variations in kidney damage in rodents according to strain and sex remain unexplained.

CHLOROFORM IN THE ENVIRONMENT

The role of $CHCl_3$ in the environment has raised much interest in the developed world since 1970. Generous funding by governmental agencies and scientific institutions, new methods of detection (gas chromatography and mass spectrophotometry) and the creation of computer-generated pharmacokinetic models have led to a large amount of research projects and publications.

1. CHCL3 IN THE OCEANS

$CHCl_3$ measurements in the oceans are complicated by its rapid evaporation and variations due to sites, seasons and temperatures. World oceans are estimated to contain 300 to 400 tons of the compound. Its main producers are the algae and seaweeds and, to a much smaller degree, the ocean floor vents and marine volcanoes and, in the littoral areas, rivers, moors and peatland. Oceans have no detoxifying mechanisms and get rid of their $CHCl_3$ through evaporation. Part of that $CHCl_3$ vapor then returns in the rain water.

2.CHCl3 IN THE ATMOSPHERE

The U.S. atmosphere contains 2 to 4 ppt (parts per trillion) to 2-4 ppb (parts per billion) CHCl3. These large variations are due to methods of titration and seasonal and geographical changes. CHCl3 in the air originates from natural and man-made sources:

A. NATURAL SOURCES: most atmospheric CHCl3 derives from its evaporation from oceans and primitive lakes, rivers, moors and savannas. Minor sources include insect colonies (ants, termites), volcanic sulfuric gases and bacterial decomposition of fecal methane.

B.MAN-MADE SOURCES: humans annually release 250,000 tons CHCl3 in the atmosphere; this is 10 to 20% of the total CHCl3 released in the air. The major contributors include:

1.Evaporation from chlorinated waters (lawns, golf courses, street cleaning, polluted lakes and rivers.

2. Refuse from pulp and paper processing plants which use chlorine or chlorine dioxide to treat their wood and cellulose products.

3.Cooling waters of energy producing plants which chlorinate their waters to prevent the growth of aqueous organisms. Chlorination of the humic content of this raw water produces $CHCl_3$ (see below)

4.Managed crop and forest fires also release small amounts of $CHCl_3$. So do incinerators, sanitary landfills and waste disposal plants.

5. Cars also emit $CHCl_3$, especially when using gasoline containing the lead-scavenging dichloromethane.

6. The industrial trichloroethylene and carbon tetrachloride accidentally released outdoors is destroyed by the atmospheric sun light or soil bacteriae and may form $CHCl_3$.

As mentioned above, the plants handling CHCl3 release very little of it outdoors.

CHCl3 is mostly removed from the atmosphere through the rain. Rain water in the developed world may contain up to 0.125 mg/l CHCl3. The latter is then re-evaporated from the soil and the surface waters. The free .OH radicals in the troposphere reduce CHCl3 to C0Cl2 and HCl which in their turn are dechlorinated and may form new CHCl3. This reduction is potentiated by smog, which is rich in free .OH radicals. Small amounts of CHCl3 are also oxidized by U.V. rays. Remaining traces persist for long periods (half life of 6 months) and travel over large distances. Minute quantities migrate into the stratosphere but do not deplete the ozone and thus do not contribute to the global warming, as recognized by the Kyoto and Montreal protocols.

3.CHCL3 IN SURFACE AND GROUND WATER

Minute amounts of CHCl3 have been found in the surface waters of primitive areas. It is the enzymatic product of algae and aquatic plants. Much larger quantities exist in the lakes, rivers and wells of

industrialized countries, the result of spillage of chlorinated urban waters, industrial plants and hazardous waste disposal sites. Even rural wells collect $CHCl_3$ from seeping urban waters and agricultural pesticides and fumigants.

Most of that aqueous $CHCl_3$ passes into the atmosphere but a small amount is enzymatically destroyed by the aquatic flora or undergoes dechlorination by OH radicals or direct oxidative photolysis.

Fishes do not bio-accumulate $CHCl_3$ and thus are safe for human consumption.

4.$CHCL_3$ IN SOIL

Over 200 tons of $CHCl_3$ are estimated to exist in the world' soil. Most of it originates from human pollution but there exist a few natural sources:

A. Rain falls (mainly)

B. Reaction of humic material with the fungal, enzymatically produced H202 and the ubiquitous Cl- ions. A large supply of humic matters are the putrified tree debris of hot, wet areas.

The CHCl3 present in the pores of the soil's upper layers is quickly evaporated. A small portion is dechlorinated by methagenic bacteriae or oxidized with resulting C02 which is released into the air. In deep layers, CHCl3 persist for long periods and may travel over large distances, thus contributing to well pollution.

HUMAN EXPOSURE TO CHCL

1.FOODSTUFFS

CHCl3 is present in many foodstuffs of developed countries, especially in processed and frozen food. The highest content is found in wheat and dairy products, vegetables, natural oils and soft drinks. Americans are estimated to consume 10 mcg CHCl3 in their daily food..

This CHCl3 originates from the soil (milk, vegetables, oils) or from treatment with chlorinated water (soft drinks, frozen or processed food). Minute amounts (1ng/l) have been found in the milk of American nursing mothers.

2.DRINKING WATER

The main source of human exposure to CHCl3 in modern countries is the chlorinated tap water. Over 170 million Americans in 1999 used that water.

Chlorination of drinking water, started at the turn of the 20th century. Despite its drawbacks and searches for better alternatives, it remains the cheapest and most efficient and practical method to disinfect water. A mixture of reactive chlorine compounds (Cl2, Na0Cl, Ca0Cl2, NHCl) is added to raw water, releasing H0Cl and OCl ions which oxidize the microorganisms. Unfortunately these chlorine compounds react with humic and flavic material present in these natural waters and produce trihalomethanes, the most significant of which is CHCl3 (JJ Rook, TA Bellar). Attempts are now made to filtrate out this organic

material before chlorination, thus reducing the amount of needed chlorine and the trihalomethane production.

Humans are exposed to chlorinated tap water in various ways:

1. Drinking: U.S. tap water may contain up to 1 mcg/l $CHCl_3$. A person drinking 2 liters of water a day thus ingests 2mcg $CHCl_3$ daily. Although not directly toxic, this dose has been suspected of being carcinogenic (see below).

2. Actual measurements and theoretical kinetic models show significant absorption of $CHCl_3$ during showers and baths with city water. A shower stall may contain 50 to 80 mg/m3 $ChCl_3$.Doses absorbed through the skin may equal these inhaled. High concentrations of $CHCl_3$ have also been found in the air and water of heated, indoor swimming pools with significant skin and lung absorption. Plasma levels of competitive swimmers may reach 0.82 mcg/l, although this falls to zero shortly after exposure. The problem does not exist with outdoor pools.

3. The indoor air of American and European houses contains 2 to 3 ppm CHCl3; this is about 25 fold the concentration found outdoors in the same areas. This indoor CHCl3 emanates from kitchen sinks, dish and cloth washers, humidifiers, and, to a small degree, bleaching powders, air purifiers, insecticides and pesticides.

4. Outdoor CHCl3: CHCl3 has consistently been found in the atmosphere of large cities and, although to a smaller degree, in that of rural areas. This CHCl3 originates from soiil and surface water evaporation, car emissions, fires, and releases from landfills.

CHCL3 AS A MUTAGEN

The mutagenic potential of CHCl3 has been extensively studied in vitro and in vivo over the past 40 years. With a few exceptions, these studies have failed to show a mutagenic effect despite occasional findings of strong affinity of the metabolite C0Cl2 for the nuclear macromolecules.

CHCL3 AS A CARCINOGEN

A. Rodents and dogs ingesting large doses of CHCl3 diluted in oil over long periods develop hepatic and renal carcinomas. There exist large differences in the resulting tumors according to species, strain, and sex. These tumors largely develop from the regenerating cells of necrosed tissues, suggesting that non-cytotoxic doses of CHCl3 may not be carcinogenic. Because of the large doses, long periods of administration and species variations these animal findings cannot be extrapolated to man

B. At least 22 epidemiological surveys, most of them done by the U.S. Environmental Protection Agency, have explored the correlation between drinking water consumption and human cancer. Several of these studies suggest a small correlation between drinking water and bladder and, to a lesser degree, colon and rectum carcinomas. The role of CHCl3 was not isolated from that of the other triahlomethanes. These studies have serious methodological flaws and better studies are needed. It thus seems that human consumption of tap water in the

U.S., with a daily intake of less than 100 mcg/kg, has not been proven to be carcinogenic.

CHCL3 AND REPRODUCTION

A. Pregnant rodents inhaling or, especially, ingesting large doses of CHCl3 show slowed fetal growth, decreased litter size and birth weight, and occasional fetal malformations. It is unclear whether this is a direct fetal effect or an indirect one through mother toxicity. Because of flaws in design and the large doses of CHCl3 administered, the results of these studies cannot be applied to man.

B. Fifteen human surveys correlating drinking water and gestational damage suggest a slight decrease in neonatal weight, a small increase in abortions and still-births and rare fetal neurological defects. These studies ignore confounding factors and have other flaws. Female technicians exposed to CHCl3 (but also other) solvents in a Swedish medical laboratory had a small increase in spontaneous abortions. The danger of CHCl3 on the human reproduction has thus not been proven.

CONCLUSIONS

Despite no longer begin used as a general anesthetic, chloroform has been extensively studied in other contexts. This paper has review what is known about many of them. Although CHCl3 is ubiquitous in our environment and has been extensively investigated, its toxicity to man thus far remains unproven.

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